

Summary of Research

Effects of Gravity on Cell Movement and Development

NASA Grant NAG2-1197
5/1/1998 – 1/31/2002

Yu-li Wang, Ph.D. Professor of Physiology

University of Massachusetts Medical School
55 Lake Avenue North
Worcester, MA 01655

The main purpose of this project was to understand how the migration and growth of cultured cells respond to mechanical forces. We have made significant progress on all the proposed aims. The most important discoveries are that changes in the environmental mechanical input, such as during space flight, can induce profound changes in cell migration, growth, and programmed cell death. In addition, using genetically engineered cells, we have gained important insight into the molecular mechanism underlying such mechanosensing processes. The results are summarized below.

I. Cell Migration Is Guided by Mechanical Signals (Lo et al., *Biophys. J.* 79:144-152)

Directional cell locomotion is critical in many physiological processes, including morphogenesis, the immune response and wound healing. It is well known that in these processes cell movements can be guided by gradients of various chemical signals. In this study, we demonstrate that cell movement can also be guided by purely physical interactions at the cell-substrate interface. We cultured NIH 3T3 fibroblasts on flexible polyacrylamide sheets coated with type I collagen. A transition in rigidity was introduced in the central region of the sheet by a discontinuity in the concentration of the bis-acrylamide crosslinker. Cells approaching the transition region from the soft side easily migrated across the boundary, with a concurrent increase in spreading area and traction forces. In contrast, cells migrating from the stiff side turned around or retracted as they reached the boundary. We call this preference for stiff substrates “durotaxis”. In addition to substrate rigidity, we discovered that cell movement is also guided by manipulating the flexible substrate with a microneedle to produce mechanical strains. Cells migrated toward pulling forces, by expanding a lateral protrusion into a lamellipodium, and away from pushing forces, by retracting lamellipodia that extended toward the needle. We conclude that changes in tissue rigidity and strain may play an important controlling role in a number of normal and pathological processes involving cell locomotion.

II. Cell Growth and Apoptosis Are Regulated by Mechanical Signals (Wang et al., *Am. J. Physiol.* 279:C1345)

We cultured NIH 3T3 cells on flexible collagen-coated polyacrylamide substrates with similar chemical properties but different rigidity. Compared to isolated cells cultured on stiff substrates, those on flexible substrates showed a decrease in the rate of DNA synthesis and an increase in the rate of programmed cell death (apoptosis). In addition, cells on soft substrates formed tissue-like aggregates, in which the rates of both growth and apoptosis were lower as compared to isolated cells. Our results suggest that cells are capable of probing substrate rigidity, and that mechanical feedback from the substrate and from neighboring cells plays an important role in regulating cell growth and survival.

III. Phagocytosis Is Modulated by Mechanical Properties of the Target (Beningo and Wang, *J. Cell Sci.* 115:849)

Phagocytosis (engulfment of micro-sized particles) by macrophages is an essential component of the immunological response and is also necessary for tissue remodeling and repair. It is commonly assumed that the selection of targets is based solely upon receptor-ligand binding. However, using polyacrylamide particles of identical chemical properties but different rigidity, we found that mechanical parameters of the target can dramatically affect the efficiency of phagocytosis. Macrophages showed a strong preference to engulf rigid objects. Furthermore, phagocytosis of soft particles can be stimulated by incubating the beads with lysophosphatidic acid

(LPA, an activator of small GTPases) and by microinjecting the cells with dominantly active Rac (a small GTPase involved in signaling the cytoskeleton), suggesting that Rac may be involved in the detection of target rigidity. These data indicate that mechanosensing plays an important role in regulating the efficiency of Fc-mediated phagocytosis and that the small GTPase Rac plays a key role in this process.

IV. Focal Adhesion Kinase (FAK) Is Involved in Mechanosensing during Fibroblast Migration (Wang et al. *Proc. Natl. Acad. Sci. USA.* 98:11295)

Focal adhesion kinase (FAK) is a non-receptor protein tyrosine kinase localized to cell-substrate adhesion sites, and is suspected to play a role in mechanosensing. In collaboration with Steve Hanks at Vanderbilt University, we addressed the possibility that FAK might be involved in the guidance of cell migration, using fibroblasts derived from FAK knockout mouse embryos and transfected with the FAK gene under the control of the tetracycline repression system to achieve inducible re-expression of FAK. Cells were cultured on flexible, collagen-coated polyacrylamide substrates for the detection of traction forces and for the application of mechanical stimulation. FAK null fibroblasts showed no decrease in traction forces on soft substrates as compared to those on stiff substrates, in contrast to FAK-expressing cells. Furthermore, compared to FAK-expressing cells, FAK null cells showed a decrease in both the migration speed and persistence. Most significantly, the migration of FAK null cells was not responsive to either substrate flexibility or mechanical stimulation. Our results suggest that FAK is involved in the detection and/or responses of migrating cells to mechanical input, possibly by converting external mechanical signals into cytoplasmic chemical events.

V. Myosin II-B Is Required for the Stability and Guidance of Cell Migration (Lo et al., in preparation)

Although myosin II is involved in the generation of traction forces in cultured fibroblasts, little is known about the role of various myosin II isoforms in cell migration. In collaboration with Bob Adelstein's group at NIH, we have approached this problem by analyzing the movements and mechanical characteristics of fibroblasts from mouse embryos where non-muscle myosin II-B was ablated by targeted gene disruption. Fibroblasts were cultured on either coverglass or on flexible polyacrylamide substrate coated with type I collagen. Cells from the wild type littermates were used as the control. Compared with control cells, myosin II-B mutant cells displayed unstable and disorganized lamellipodia. Analysis of the cell migration pattern indicated an increase in speed and decrease in persistence. These changes lead to defects in directional movement, as demonstrated with both Boyden chamber and wound healing assays. In addition, wild type cells migrated toward rigid substrates (durotaxis) or stretching forces, while mutant cells showed impaired responses to these mechanical signals. Furthermore, mutant cells exerted only a slight reduction in traction forces on the substrate than did wild type cells, but lost their ability to respond to substrate rigidity or to applied mechanical forces. These results suggest that myosin II-B is involved not in the generation of traction forces, but in mechanosensing and in directional cell movement. We propose that a myosin II-B-based sensing mechanism physically probes the substrate and converts the response into chemical signals such as tyrosine phosphorylation.

VI. Publications

Lo, C.-M., Wang, H.-B., Dembo, M. and Wang, Y.-L. (2000) Cell movement is guided by the rigidity of the substrates. *Biophys. J.* 79:144-152.

Wang, H.-B., Dembo, M. and Wang, Y.-L. (2000) Substrate flexibility regulates growth and apoptosis of normal but not transformed cells. *Am. J. Physiol.* 279:C1345-C1350.

Wang, H.-B., Dembo, M., Hanks, S.K. and Wang, Y.-L. (2001) Focal adhesion kinase is involved in mechanosensing during fibroblast migration. *Proc. Natl. Acad. Sci. USA* 98:11295-11300.

Beningo, K.A. and Wang, Y.-L. (2002) Fc-receptor mediated phagocytosis is regulated by mechanical properties of the target. *J. Cell Sci.* 115:849-856.

Beningo, K.A. and Wang, Y.-L. (2002) Flexible substrata for the detection of traction forces. *Trends Cell Biol.* 12:79-84.

Beningo, K.A., Lo, C.-M. and Wang, Y.-L. (2002) Flexible polyacrylamide substrata for the analysis of mechanical interactions at cell-substratum adhesions. *Methods Cell Biol.*, in press.

VII. Invention

None